

Identification of a serpin-enzyme complex receptor on human hepatoma cells and human monocytes

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ABSTRACT Formation of the covalently stabilized complex of α_1 -antitrypsin (α_1 -AT) with neutrophil elastase, the archetype of serine proteinase inhibitor serpin-enzyme complexes, is associated with structural rearrangement of the α_1 -AT molecule and hydrolysis of a reactive-site peptide bond. An \approx 4-kDa carboxyl-terminal cleavage fragment is generated. α_1 -AT-elastase complexes are biologically active, possessing chemotactic activity and mediating increases in expression of the α_1 -AT gene in human monocytes and macrophages. This suggested that structural rearrangement of the α_1 -AT molecule, during formation of a complex with elastase, exposes a domain that is recognized by a specific cell surface receptor or receptors. To test this hypothesis, the known three-dimensional structure of α_1 -AT and comparisons of the primary structures of the serpins were used to select a potentially exteriorly exposed and highly conserved region in the complexed form of α_1 -AT as a candidate ligand (carboxyl-terminal fragment, amino acids 359–374). We show here that synthetic peptides based on the sequence of this region bind specifically and saturably to human hepatoma cells and human monocytes ($K_d = 4.0 \times 10^{-8}$ M, 4.5×10^5 plasma membrane receptors per cell) and mediate increases in synthesis of α_1 -AT. Binding of peptide 105Y (Ser-Ile-Pro-Pro-Glu-Val-Lys-Phe-Asn-Lys-Pro-Phe-Val-Tyr-Leu-Ile) is blocked by α_1 -AT-elastase complexes, antithrombin III (AT III)-thrombin complexes, α_1 -antichymotrypsin (α_1 -ACT)-cathepsin G complexes, and, to a lesser extent, complement component C1 inhibitor-C1s complexes, but not by the corresponding native proteins. Binding of peptide 105Y is also blocked by peptides with sequence corresponding to carboxyl-terminal fragments of the serpins AT III and α_1 -ACT, but not by peptides having the sequence of the extreme amino terminus of α_1 -AT. The results also show that peptide 105Y inhibits binding of ¹²⁵I-labeled α_1 -AT-elastase complexes. Thus, these studies demonstrate an abundant, relatively high-affinity cell surface receptor which recognizes serpin-enzyme complexes (SEC receptor). This receptor is capable of modulating the production of at least one of the serpins, α_1 -AT. Since the ligand specificity is similar to that previously described for *in vivo* clearance of serpin-enzyme complexes, the SEC receptor may also be involved in the clearance of certain serpin-enzyme complexes.

α_1 -Antitrypsin (α_1 -AT) is a 55-kDa serum glycoprotein that inactivates neutrophil elastase. Since neutrophil elastase is capable of degrading most of the constituents of connective tissue matrices, its inhibitor α_1 -AT is thought to play a critical role in connective tissue turnover in homeostasis and tissue injury/inflammation (reviewed in refs. 1–3).

α_1 -AT is the archetype of the serine proteinase inhibitor (serpin) family, which includes antithrombin III (AT III), α_1 -antichymotrypsin (α_1 -ACT), complement component C1

inhibitor, heparin cofactor II (HC II), α_2 -antiplasmin, protein C inhibitor, plasminogen activator inhibitors I and II, and protease nexins I and II (reviewed in ref. 4). Although there is only 25–30% primary sequence homology among the members of this family, there is a much greater degree of functional similarity and a similar mechanism of action. Each serpin binds its target enzyme at a substrate-like region within the carboxyl-terminal portion of the molecule. The enzyme is inactivated as a covalently stabilized enzyme-inhibitor complex is formed. During complex formation there is also structural rearrangement of the inhibitor and hydrolysis of the reactive-site peptide bond. However, it is not yet known whether hydrolysis of the reactive-site peptide bond goes to completion or whether the carboxyl-terminal fragment is dissociated from the macromolecular complex (reviewed in ref. 3).

Several recent studies suggest that α_1 -AT-elastase complexes have intrinsic functional activities. These complexes stimulate neutrophil chemotaxis (5) and mediate increases in expression of the α_1 -AT gene in human monocytes and macrophages (6, 7). α_1 -AT-elastase complexes are more rapidly cleared from the circulation than the corresponding native proteins. In fact, *in vivo* clearance of α_1 -AT-elastase complexes is blocked by other serpin-enzyme complexes (8, 9), suggesting the presence of a common recognition system. Taken together, these observations suggest that structural rearrangement of the α_1 -AT molecule, during formation of a complex with elastase, exposes a domain that is recognized by a specific cell surface receptor, or receptors. In the following study we used synthetic peptides based on the sequence of the carboxyl-terminal fragment of α_1 -AT as candidate mediators for regulation of α_1 -AT synthesis and as candidate ligands for cell surface binding. This region of the α_1 -AT molecule was selected because it had been previously implicated in the chemotactic activity of α_1 -AT-elastase complexes (5) and because crystal structure analysis (10) predicted that a domain within this region was exteriorly exposed after formation of a complex.

EXPERIMENTAL PROCEDURES

Materials. Peptides were synthesized by the solid-phase method (11, 12), purified, and subjected to amino acid composition and sequence analysis. Peptides were dissolved in water and then added to cell culture fluid. Preparation of purified human plasma α_1 -AT, leukocyte elastase, and leukocyte cathepsin G has been previously described (6). Purified human plasma AT III and α_1 -ACT were purchased from Athens Research and Technology (Athens, GA). Purified human thrombin was purchased from Boehringer-Mann-

Abbreviations: α_1 -AT, α_1 -antitrypsin; AT III, antithrombin III; α_1 -ACT, α_1 -antichymotrypsin; serpin, serine proteinase inhibitor; SEC, serpin-enzyme complex.

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heim. Human complement component C1s was a gift from David Bing (Boston, MA) and C1 inhibitor was from Alvin E. Davis (Boston, MA). Recombinant yeast-derived [Val³⁵⁸] α_1 -AT was kindly provided by I. Bathurst and P. Barr (Emeryville, CA).

Cell Culture. Confluent monolayers of human blood monocytes from normal individuals were established by a previously described technique (13). HepG2 cells were maintained in culture as previously described (13).

Metabolic Labeling. Confluent monolayers were rinsed and incubated at 37°C in the presence of medium containing [³⁵S]methionine at 250 μ Ci/ml (1 Ci = 37 GBq). To determine the net synthesis of α_1 -AT and a control protein, factor B, cells were subjected to a short pulse (20 min) and radiolabeled α_1 -AT or factor B was assayed in the cell lysates. Solubilization of cells, clarification of cell lysates after labeling, assay of total protein synthesis, immunoprecipitation, and SDS-PAGE have been described (13–15).

Determination of Cell Surface Receptor Binding. Peptide 105Y was labeled with ¹²⁵I by using chloramine T and was purified by gel filtration on Bio-Gel P2 (Bio-Rad). The specific radioactivity of various preparations was 1500–3000 cpm/ng of peptide. Recombinant [Val³⁵⁸] α_1 -AT was labeled with ¹²⁵I by using Iodobeads (Pierce) and purified by gel filtration through Sephadex G-10 (Pharmacia). The specific radioactivity was 1000–5000 cpm/ng of protein. For binding studies, cells were washed with Dulbecco's phosphate-buffered saline and incubated at 4°C for 2 hr with ¹²⁵I-labeled peptide 105Y (¹²⁵I-peptide 105Y) or [Val³⁵⁸] α_1 -AT diluted in Dulbecco's modified Eagle's medium containing 10 mM HEPES and cytochrome *c* at 0.1 mg/ml. The cells were then rinsed in phosphate-buffered saline and homogenized in 1 M NaOH, and cell-associated radioactivity was determined. Specific binding of iodinated ligand was defined as the difference between total and nonspecific binding. Nonspecific binding was determined by the addition of a 200-fold excess of unlabeled ligand. Scatchard plot analysis of equilibrium binding data was performed as previously described (16, 17).

RESULTS

Effects of Synthetic Peptides on Synthesis of α_1 -AT. Crystal structure analysis has predicted that residues 359–365 of the post-complex form of α_1 -AT are particularly well exposed at the surface of the molecule (10). These residues are adjacent to a region which is highly conserved among the serpin family (12). We synthesized several peptides overlapping the sequence of this region (Fig. 1a) and examined the effect of each peptide on synthesis of α_1 -AT in human monocytes (Fig. 1b and c). Separate monolayers of monocytes were incubated at 37°C for 10 hr in serum-free control medium or medium supplemented with peptide 113 at several different concentrations (Fig. 1b). As a positive control, a separate monocyte monolayer was incubated with human neutrophil elastase. We have previously shown that elastase mediates an increase in synthesis of α_1 -AT in monocytes, probably by forming a complex with endogenous monocyte α_1 -AT (6, 7). Cells were then labeled for 20 min with [³⁵S]methionine to determine net synthesis of α_1 -AT. Peptide 113 mediates a concentration-dependent increase in synthesis of α_1 -AT. The magnitude of the effect is comparable to that of exogenous elastase (lane 2) or α_1 -AT-elastase complexes (6). Peptides 109 and 105 also mediate concentration-dependent increases in α_1 -AT synthesis (Fig. 1c). A peptide based on the extreme amino-terminal sequence of α_1 -AT (Nterm) has no effect on synthesis of α_1 -AT. The positive effect of peptides 109 and 105 was demonstrated in the presence of polymyxin B at concentrations which abrogate the known effect (13) of lipopolysaccharide on α_1 -AT synthesis (lanes 3 and 4). It should be noted that two radiolabeled polypeptides, \approx 48 and 52 kD, are immunoprecipitated from the cell lysates in Fig. 1c. The

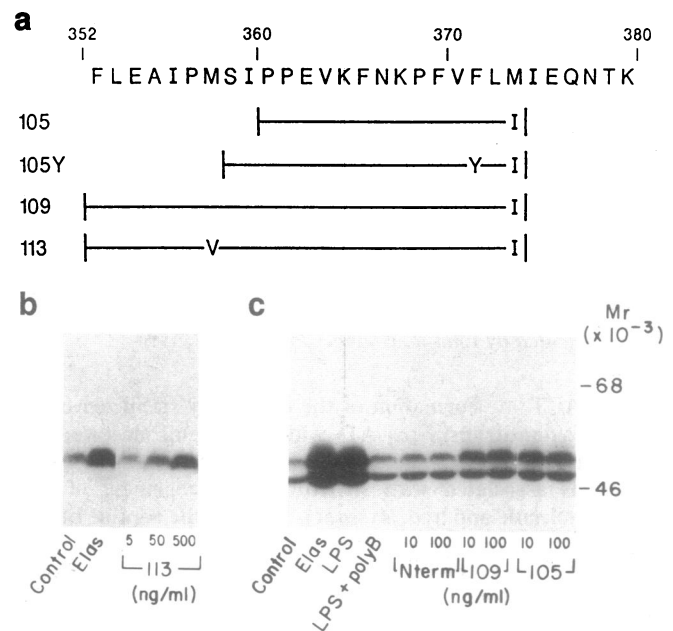


FIG. 1. Effect of synthetic peptides on synthesis of α_1 -AT in human monocytes. (a) Amino acid sequences of α_1 -AT and several synthetic peptides. (b) Monolayers of peripheral blood monocytes were incubated for 10 hr at 37°C in control serum-free medium, medium supplemented with human neutrophil elastase (50 ng/ml), or medium supplemented with peptide 113 in the specified concentrations. (c) Monocytes were incubated for 10 hr at 37°C in control serum-free medium, medium supplemented with human neutrophil elastase (50 ng/ml), *Escherichia coli* lipopolysaccharide (LPS; 100 ng/ml), LPS and polymyxin B (15 μ g/ml), or synthetic peptides in the specified concentrations together with polymyxin B. Cells were then subjected to metabolic labeling, and radiolabeled cell lysates were subjected to immunoprecipitation with anti-human α_1 -AT followed by SDS/PAGE/fluorography. The sequence of peptide Nterm is EDPOGDAAQ. Molecular weight markers are indicated at the right margin.

additional \approx 48-kD polypeptide is a specific α_1 -AT polypeptide (18) but its concentration varies among donors (data not shown). The effect of the synthetic peptides on synthesis of α_1 -AT is specific in that there is no change in total trichloroacetic acid-precipitable protein synthesis and no change in the synthesis of another specific secretory protein of monocytes, complement protein factor B (data not shown).

We next examined the effect of synthetic peptide 105Y on the synthesis of α_1 -AT in the human hepatoma cell line HepG2 (Fig. 2). HepG2 cells were incubated at 37°C for 5 hr in serum-free control medium or medium supplemented with peptide 105Y at two different concentrations. The previously described methods were used to determine net synthesis of α_1 -AT. Peptide 105Y mediates a concentration-dependent increase in synthesis of α_1 -AT (Fig. 2 Left) but has no effect on the control protein factor B (\approx 90 kDa; Fig. 2 Right). The differences in the relative biological activity of these peptides in HepG2 cells and monocytes (effective concentration in monocytes \approx 1/10th to 1/100th that in HepG2 cells) may reflect the stability of peptides during a 5- to 10-hr incubation at 37°C. In any case, peptides based on the α_1 -AT sequence 359–374 have positive regulatory effects on α_1 -AT synthesis in two different cell types.

Cell Surface Binding of Synthetic Peptides. As the shortest peptide with biological activity and, therefore, the easiest to synthesize and modify, peptide 105 was selected for further studies. The amino terminus was extended to the P₁' serine residue and phenylalanine-372 was replaced by tyrosine for radioiodination [peptide 105Y (Fig. 1a)]. This substitution did not eliminate biological activity of the peptide as shown by its

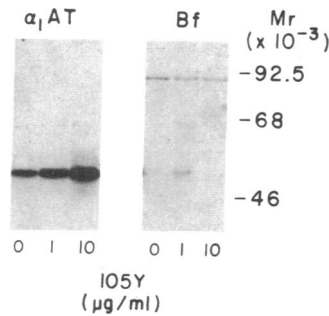


FIG. 2. Effect of synthetic peptide 105Y on synthesis of α_1 -AT in HepG2 cells. HepG2 cells were incubated for 5 hr at 37°C with serum-free control medium or medium supplemented with 105Y in the specified concentrations. Cells were then rinsed and subjected to metabolic labeling, and radiolabeled cell lysates were subjected to sequential immunoprecipitation with anti-human α_1 -AT (Left) and then with anti-human factor B (Right). Immunoprecipitates were subjected to SDS/PAGE/fluorography.

positive regulatory effect on α_1 -AT synthesis in Fig. 2. The presence of a positive regulatory effect in HepG2 cells provides a well-characterized continuous model cell culture system for these binding studies. Separate monolayers of HepG2 cells were incubated for 2 hr at 4°C with several different concentrations of 125 I-peptide 105Y in the absence or presence of unlabeled peptide 105Y in 200-fold molar excess (Fig. 3). There is specific and saturable binding. Scatchard plot analysis indicates a K_d of 4.0×10^{-8} M and approximately 4.5×10^5 plasma membrane receptors per cell. The data are consistent with a single class of binding sites on these cells. There is also specific, saturable binding of 125 I-peptide 105Y to monocytes (data not shown). 125 I-peptide 105Y does not bind to human hepatoma Hep3B cells or to the human intestinal epithelial cell line Caco2 or to human diploid fibroblasts (data not shown). There is no competition for binding of 125 I-peptide 105Y by purified proteins that constitute ligands for other known hepatocyte receptors, including asialoorosomuroid, diferric transferrin, insulin, epidermal growth factor, interleukin-1 β , interleukin-6, methylamine-treated α_2 -macroglobulin, or by the peptide f-Met-Leu-Phe (data not shown). There is time-dependent and temperature-dependent dissociation of peptide 105Y from HepG2 cells (data not shown).

We also examined the specificity of 125 I-peptide 105Y binding by competition with other synthetic peptides (Fig. 4). HepG2 cells were incubated for 2 hr at 4°C with 125 I-peptide 105Y in the absence or presence of other synthetic peptides, unlabeled. Peptides 210 and 96, which overlap amino- and carboxyl-terminal regions of 105Y, each compete for binding of 105Y to HepG2 cells. These data indicate that both amino- and carboxyl-terminal portions of peptide 105Y contribute to ligand binding. Other serpins contain regions of highly homologous amino acid sequence in their carboxyl-terminal fragments (12). Peptides corresponding to sequences from two of these, α_1 -ACT (peptide 10) and AT III (peptide 85), were synthesized and examined in this assay. Peptide 85 is actually a hybrid with α_1 -AT 355–362 and AT III 393–402 sequences (Fig. 4a). Binding of 105Y is blocked by peptides 10 and 85 but not by a peptide corresponding to α_1 -AT amino-terminal residues 1–10 (Fig. 4b).

Next, we examined the ability of unlabeled serpins in native and complex form to compete for binding of 125 I-peptide 105Y (Fig. 5). Binding of radioiodinated peptide 105Y was not blocked by native α_1 -AT or native leukocyte elastase, but it was blocked in a concentration-dependent manner by a reaction mixture containing α_1 -AT-elastase complexes (Fig. 5a). There is, at most, a 2-fold difference in the capacity of unlabeled α_1 -AT-elastase complexes and

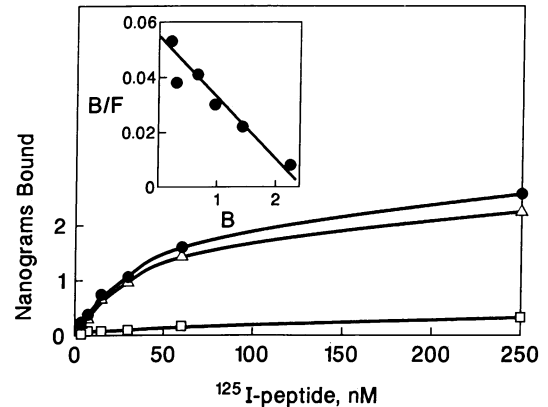


FIG. 3. Specific binding of 125 I-peptide 105Y to HepG2 cells. Cells were incubated for 2 hr at 4°C with several different concentrations of 125 I-peptide 105Y in the absence (●, total ligand binding) or presence of unlabeled peptide 105Y in 200-fold molar excess (□, nonspecific binding). The cells were then rinsed and cell-associated radioactivity was determined by γ counting of cell homogenates. Specific ligand binding is shown with Δ . In the Scatchard plot analysis (Inset), B represents ng bound per 10^6 cells and F represents free peptide in nM.

unlabeled peptide to compete for this binding site. However, it is not possible to determine whether the difference is real or apparent, since the actual concentration of ligand presented to the binding site by the reaction mixture containing α_1 -AT-elastase complexes cannot be measured.

Binding of 125 I-peptide 105Y is also blocked by complexes of AT III-thrombin, α_1 -ACT-cathepsin G, and, to a lesser extent, C1 inhibitor-C1s, but not by the corresponding native proteins (Fig. 5b). These data indicate that multiple serpin-

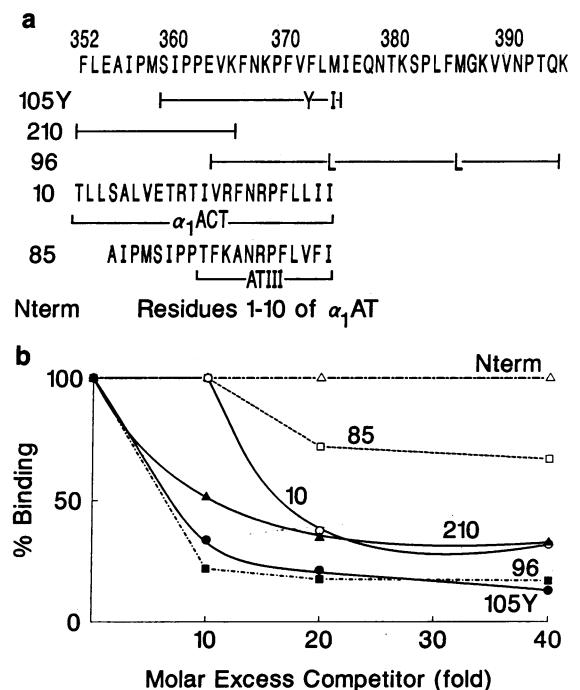


FIG. 4. Inhibition of 125 I-peptide 105Y binding to HepG2 cells by other synthetic peptides. (a) Amino acid sequence of α_1 -AT and several synthetic peptides. (b) Confluent monolayers of HepG2 cells were incubated for 2 hr at 4°C with subsaturating concentrations of 125 I-peptide 105Y (50 nM) in the absence or presence of various excess amounts of peptides Nterm (Δ), 85 (\square), 210 (\blacktriangle), 10 (\circ), 96 (\blacksquare), or 105Y (\bullet). Cell-associated radioactivity was determined and expressed on the vertical axis as percent binding. Each point represents the average of duplicate determinations.

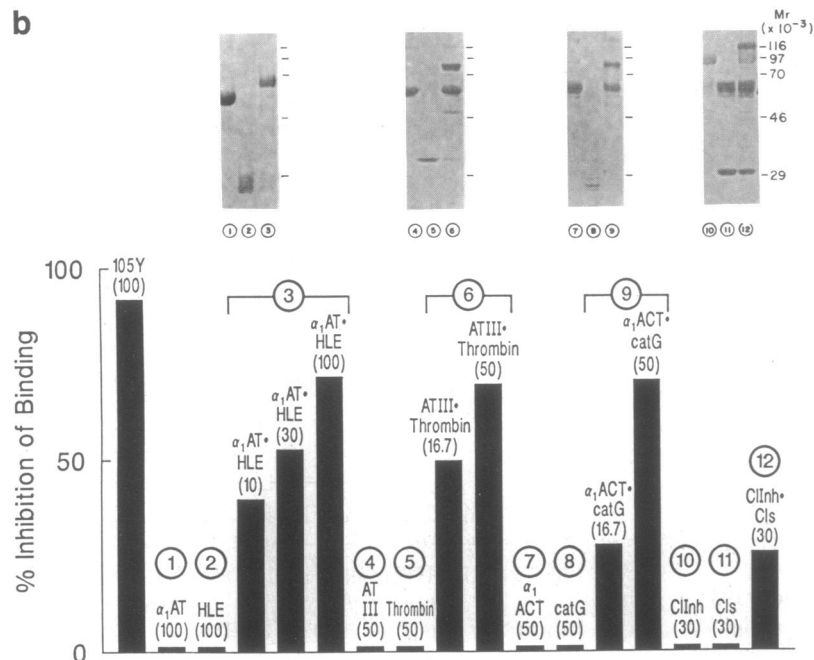
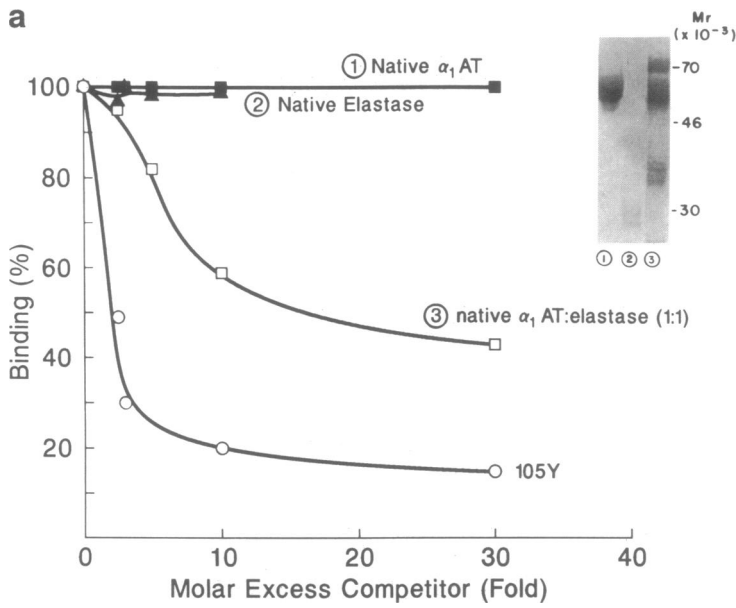


FIG. 5. Inhibition of ¹²⁵I-peptide 105Y binding to HepG2 cells by purified proteins. Confluent monolayers of HepG2 cells were incubated for 2 hr at 4°C with subsaturating concentrations of ¹²⁵I-peptide 105Y (50 nM) in the absence or presence of various excess amounts of purified proteins or reaction mixtures. Cell-associated radioactivity was then determined and expressed on the vertical axis as percent binding for *a* and percent inhibition of binding for *b*. The relative amount in moles of each competitor is shown on the horizontal axis for *a* and in parentheses above each set of bars for *b*. An aliquot of each preparation of unlabeled competitor was subjected to SDS/PAGE under reducing conditions followed by staining in Coomassie brilliant blue (upper right for *a*, above each set of bars for *b*). Molecular weight markers are indicated at the right margin. In each case purified inhibitor, purified enzyme, or reaction mixtures containing inhibitor and enzyme in 1:1 molar ratio were incubated for 30 min at 37°C in 10 mM phosphate-buffered saline, pH 7.0. For AT III-thrombin and C1 inhibitor-C1s, heparin was also included in the reaction mixture at 2 units/ml. Phenylmethylsulfonyl fluoride was then added to all samples to a concentration of 2 mM and the incubation was continued for another 10 min at 37°C. HLE, human leukocyte (neutrophil) elastase; catG, cathepsin G; C1Inh, C1 inhibitor.

enzyme complexes are recognized by the binding site. An alignment of the sequences of these serpins shows a high degree of functional homology between residues 359–374 of the α_1 -AT sequence (12).

Cell Surface Binding of α_1 -AT-Elastase Complexes. On the basis of crystal analysis (10) it might be argued that residues close to 359 are obscured by the protease and that residues close to 374 are buried in the internal barrel structure of the α_1 -AT molecule. To further address these issues and the overall question of whether this region of the α_1 -AT-elastase complex is available for receptor binding, we examined the binding of labeled α_1 -AT-elastase complexes in the absence or presence of unlabeled peptide 105Y (Table 1). For this experiment we used recombinant yeast-derived [Val³⁵⁸] α_1 -AT. Substitution of valine for methionine makes the α_1 -AT relatively resistant to oxidative inactivation during radioiodination. [Val³⁵⁸] α_1 -AT was iodinated on tyrosines by using chloramine T. It was then allowed to react with human neutrophil elastase, and the presence of α_1 -AT-elastase complexes was demonstrated by SDS/PAGE of the reaction mixture (data not shown). The reaction mixture was then

subjected to a competitive binding assay in HepG2 cells for 2 hr at 4°C. Table 1 shows that binding of labeled α_1 -AT-elastase complexes is blocked by unlabeled α_1 -AT-elastase complexes and by unlabeled peptide 105Y but not by unlabeled native α_1 -AT and not by an unlabeled irrelevant but structurally similar peptide. The relatively high degree of nonspecific binding in these experiments is probably due to the need for an α_1 -AT-elastase reaction mixture as the labeled ligand. In other experiments we have shown that unlabeled peptide 105Y also competes for cell surface binding of labeled proteolytically modified α_1 -AT (data not shown). Together with the data presented in Fig. 4, these data provide strong functional evidence that at least part of the domain encoded by peptide 105Y is presented by α_1 -AT-elastase complexes for receptor binding. The last series of results also provides evidence that the carboxyl-terminal fragment of α_1 -AT is not freely dissociated from the complex or proteolytically modified forms of α_1 -AT. There are no tyrosines in the carboxyl-terminal fragment of α_1 -AT, so any cell-associated radioactivity is derived from binding of the larger 48-kD fragment of α_1 -AT. Unlabeled peptide 105Y must therefore compete for

Table 1. Binding of ¹²⁵I-labeled α_1 -AT- elastase complexes to HepG2 cells

Unlabeled competitor	¹²⁵ I-labeled complex bound, cpm per well			
	100 nM	50 nM	25 nM	12.5 nM
None	11,161	8992	6558	3632
α_1 -AT-elastase complexes	7,000 (37.3%)	5694 (36.7%)	3539 (46.1%)	2497 (31.3%)
Peptide 105Y	6,992 (37.4%)	5248 (41.3%)	2735 (58.3%)	1550 (57.3%)
Native α_1 -AT	10,314 (7.6%)	ND	ND	3508 (3.5%)
Irrelevant peptide*	10,900 (2.4%)	ND	ND	3292 (9.4%)

¹²⁵I-labeled α_1 -AT-elastase complex at the concentrations in the column headings was allowed to bind to HepG2 cells for 2 hr at 4°C. Unlabeled competitors were added at 30-fold molar excess. Results are reported as absolute cpm per well with percent inhibition of maximal binding in parentheses. ND, not determined.

*Irrelevant peptide is a peptide with sequence of human interleukin 1 α , residues 247–271, KQDYWVCLGGAPPSITDF-QILENQA.

binding of a molecule from which the carboxyl-terminal fragment is not freely dissociated.

DISCUSSION

These data provide evidence for an abundant, high-affinity cell surface receptor which recognizes α_1 -AT-elastase and other serpin-enzyme complexes (SEC receptor). The SEC receptor activates a signal transduction pathway for regulation of α_1 -AT gene expression and, therefore, modulates the net elastase/anti-elastase balance in the local microenvironment. The results of the current study also suggest that this receptor-linked signal transduction pathway is present in hepatocytes as well as in cells of mononuclear phagocyte lineage. This was not predicted by previous studies in which the effect of elastase on α_1 -AT synthesis in human hepatoma cells was examined (6). It is possible that much higher concentrations of elastase are necessary in this cell type to generate optimal ratios for the formation of endogenous α_1 -AT-exogenous elastase complexes.

Characterization of the α_1 -AT 359–374 domain in the current study indicates that it meets several critical requirements for designation of it as a ligand-binding domain of α_1 -AT-elastase complexes. First, a synthetic peptide based on the sequence of this domain (peptide 105Y) binds specifically and saturably to a single class of receptors on the plasma membrane of hepatoma cells and monocytes (Fig. 3). Second, peptide 105Y and other peptides based on the sequence of this domain have the same positive regulatory effect on α_1 -AT gene expression as that of α_1 -AT-elastase complexes (Figs. 1 and 2). Third, binding of peptide 105Y is blocked by α_1 -AT-elastase complexes but not by the corresponding native proteins (Fig. 5a). Fourth, unlabeled peptide 105Y blocks the binding of labeled α_1 -AT-elastase complexes (Table 1). These data also provide strong evidence that at least part of the domain encoded by peptide 105Y is presented by α_1 -AT-elastase complexes for receptor binding and militate against arguments that this domain is obscured by elastase or that critical binding determinants are buried within the internal barrel structure of the α_1 -AT-elastase macromolecular complex. It is important to note that α_1 -AT-elastase and other serpin-enzyme complexes used in our experiments, and likely to be present in complex biological fluids, represent reaction mixtures. It is highly probable that under these dynamic conditions the macromolecular complex assumes several different configurations, each or several of which could present the ligand-binding domain for cell surface receptor binding. It is also important to note that while our competitive binding data indicate that both amino- and carboxyl-terminal portions of the α_1 -AT 359–374 domain contribute to ligand binding, further studies are necessary to determine the minimal requirements for receptor binding.

The SEC receptor recognizes α_1 -AT-elastase, α_1 -ACT-cathepsin G, AT III-thrombin and, to a lesser extent, C1

inhibitor-C1s complexes (Fig. 4 and 5b). Previous studies have suggested a similar specificity for *in vivo* clearance of α_1 -AT-protease complexes; i.e., clearance of α_1 -AT-protease complexes was blocked by AT III-thrombin and α_1 -ACT-cathepsin G complexes as well as by heparin cofactor II-thrombin complexes (8, 9). These data suggest that the SEC receptor may also be involved in clearance of certain serpin-enzyme complexes. It will be of great interest to determine whether other members of the serpin family bind to this receptor and/or to other members of a family of similar receptors. Tissue-type plasminogen activator (t-PA)-plasminogen activator inhibitor I (PAI I) complexes are excellent candidates for binding to the SEC receptor, since the carboxyl-terminal fragment of PAI I bears a sequence highly homologous with α_1 -AT 364–374 and since ¹²⁵I-labeled t-PA-PAI I complexes have been reported to be internalized by HepG2 cells (19). It will also be of great interest to determine if the SEC receptor recognizes serpin family members corticosteroid- and thyroid-hormone-binding globulins and, thereby, plays a role in corticosteroid and thyroid hormone metabolism.

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